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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: **Jon A. Wolff, Vladimir S. Trubetskoy, Aaron G. Loomis, Paul M. Slattum, Sean D. Monahan, James E. Hagstrom, Vladimir G. Budker**

Examiner: **Richard A. Schnizer**

Serial No.: **09/328,975**

Filed: **06/09/1999**

Group Art Unit: **1635**

For: **Charge Reversal of Polyion Complexes**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.131

Dear Sir:

I, an inventor, Vladimir S. Trubetskoy, hereby declare as follows:

1. I am an inventor of the captioned application.
2. Photocopies of pages from my, Vladimir Trubetskoy's, personal laboratory notebook showing recharging of DNA/polycation particles beginning on December 16, 1997 accompany this Declaration.
3. It is known to me that the process performed in the notebook pages results in the formation of negatively charged tertiary complexes as described in the present specification.
4. The recharging process was conceived prior to the effective date of the Office Action prior art reference.
5. Developed of the recharging process occurred with due diligence from conception to the filing of the application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

6/7/04

Vladimir S. Trubetskoy Date

1.5 ml of react mix applied to Aldrich preparative TLC silica plate and run in $CHCl_3$: MeOH (65 20) system.

Product band was scraped off the plate.

12/16/97 Work with STDNA-bis MIS

Part of silica (above) was washed with

① $CHCl_3$

② $CHCl_3$/MeOH 65:20

Substantial amounts of DSS (upper spot is present)

Whole amount of silica was washed with

① $CHCl_3$/MeOH 65:10

② ← " " 65:30 → this fraction was evaporated

Work on recharging surface of caged DNA particles.

Caged particles are positively charged. If you add excess of polyanion it can recharge the surface to the opposite charge.

Caged particles were prepared in Bulkeley conditions (p. 72)

After 2h of incubation of react. mix at room t°

The mixture was diluted twice with deionized H_2O

and to 12 µ DNA / 48 µ PLL caged, 500 µ of polymethac-

rylic acid (pMAA) were added.

No.	FI	Conc.	
1	239.385	-10408	DNA/PLL (1:6) caged 1.7 DTBP
2	525.217	-22835	+500 µ pMAA
3	392.396	-17060	after centrifug.
4	720.091	-31308	+ 150mM NaCl
5	481.248	-20923	after centrifug.

ζ-potential was also measured

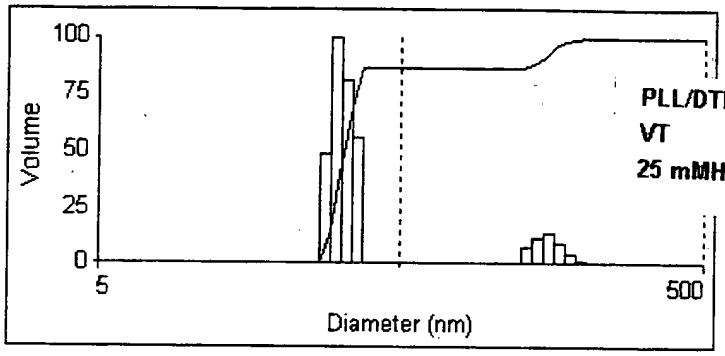
Run	Zeta Potential (mV)	Half Width (mV)
1	7.66	2.34
2	8.01	2.18
3	8.08	2.22
4	10.20	2.58
5	8.06	2.63
6	6.74	2.25
7	6.69	2.29
8	23.20	2.26
9	8.05	2.24
10	27.45	4.86
Mean	11.41	2.58
Std. Error	2.36	0.26

PLL/DTBP(6,1.7)nosalt (Run 10)
VT
,DNA=17ug/ml, 17 mM HEPES, pH 8.0

Run	Zeta Potential (mV)	Half Width (mV)
1	-29.03	2.80
2	-7.70	4.06
3	-15.37	2.74
4	-25.43	3.53
5	-53.89	2.89
6	-16.53	2.89
7	-28.26	2.63
8	-24.13	3.00
9	-26.00	7.24
10	-35.16	4.16
Mean	-26.15	3.59
Std. Error	3.97	0.44

PLL/DTBP(6,1.7)+4xpMAAnosalt (Run 10)
VT
,DNA=17ug/ml, 17 mM HEPES, pH 8.0

After addition of pMAA, I_{50} is increasing somewhat but still particle sizing



PLL/DTBP(6,1.7)rech4xpMAAsentr
VT
25 mMHEPESpH8.DNA=25ug/ml,pMAA=1 mg/ml

30.7.04/114g/150g

Basically the same effect was observed with dextran-sulfate(DS)as counterion.
The mixture was as indicated on p.75 with exception that DS was added instead of pMAA

Run	Zeta Potential (mV)	Half Width (mV)
1	33.22	2.41
2	27.98	2.61
3	20.17	3.26
4	26.99	2.22
5	10.37	2.35
6	27.01	2.06
7	33.33	2.24
8	25.83	4.46
9	28.83	2.93
10	29.39	2.18
Mean	26.31	2.67
Std. Error	2.13	0.23

PLL/DTBP(6,1.7)nosalt (Run 10)
VT
,DNA=25ug/ml, 25 mM HEPES, pH 8.0,DS=0.5mg/ml

Run	Zeta Potential (mV)	Half Width (mV)
1	-7.34	2.32
2	-22.67	2.92
3	-13.63	2.19
4	-15.95	6.56
5	-2.55	3.97
6	-21.18	2.29
7	-25.78	2.10
8	-13.92	2.42
9	-11.06	2.01
10	-15.94	5.32
Mean	-15.00	3.21
Std. Error	2.23	0.50

PLL/DTBP(6,1.7)+500ugDSnosalt (Run 10)
VT
,DNA=25ug/ml, 25 mM HEPES, pH 8.0,DS=0.5mg/ml

25 mg P-2636 Lot 75H5551

SIGMA®

POLY-L-LYSINE
Hydrobromide [25988-63-0]

CAUTION: The chemical, physical and toxicological properties of this product have not been thoroughly investigated. Exercise due care.

Desiccate

Store at less than 0°C

DP(vis)	251
MW(vis)	52,400
DP(LALLS)	252
MW(LALLS)	52,700
M_w/M_n(SEC-LALLS)	1.10

For laboratory use only. Not for drug, household or other uses. MSDS available

SIGMA CHEMICAL CO. P.O. Box 14508 St. Louis, MO 63178 USA 314-771-5750

12/17/97 Titrations of DNA/PLL (1:6) caged and non-caged with dextran sulfate.

Bridlier's solution was prepared as described in p. 72 this volume.

V = 1.5ml (30µg DNA / 114µg PLL)

50µg - 500µg of dextran sulfate (M_w = 500 kDa, Sigma) were added to each sample and I_{50}, TOTO, size and ζ-potential were measured. Some non-caged samples were prepared in the same conditions.

TOTO concentrations: (8λ of stock TOTO into 20 ml of 25 mM HEPES, pH 8.0; 10λ of sample → 0.5 ml TOTO)

Caged

No.	FI	Conc. I_{50}
1	145.344	-6319.3 0
2	395.046	-17175 50
3	1004.619	-43679 100
4	819.067	-35611 150
5	702.273	-30533 200
6	512.809	-22296 300
7	504.484	-21934 400
8	421.555	-18328 500

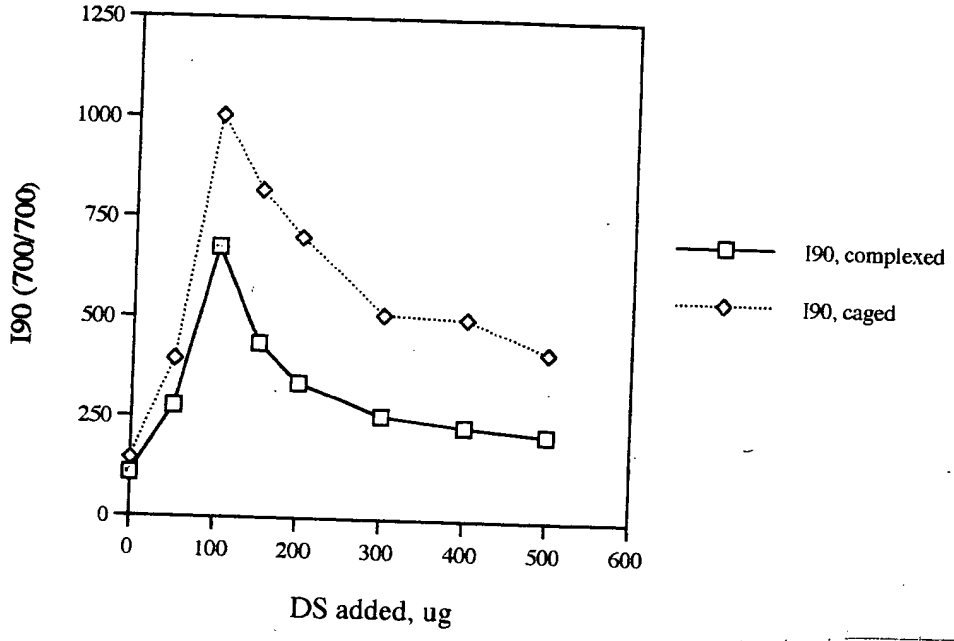
No.	FI	Conc. TOTO	
1	28.999	-1260.8	F_0
2	687.116	-29874	F_{max} 659
3	47.693	-2073.6	0 19 3%
4	38.309	-1665.6	50 10 2%
5	72.144	-3136.7	100 44 7%
6	234.264	-10185	150 206 31%
7	203.611	-8852.7	200 175 27%
8	175.301	-7621.8	300 147 22%
9	161.145	-7006.3	400 133 20%
10	160.371	-6972.7	500 132 20%

Complexed

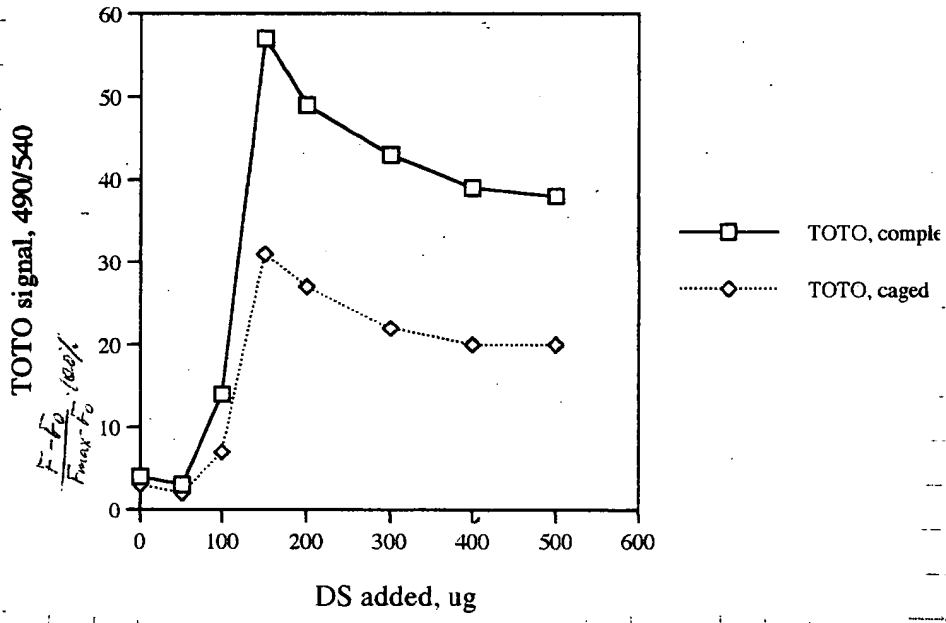
No.	FI	Conc.		
1	108.628	-4723.0		
2	278.651	-12115		
3	676.371	-29407		
4	435.570	-18937		
5	338.690	-14725		
6	258.092	-11221		
7	234.890	-10212		
8	215.716	-9379.0		

No.	FI	Conc.			
1	~~96.057~~	~~4176.4~~			
2	533.456	-23193	F_{max}	490	
3	64.342	-2797.5	0	24	4%
4	60.599	-2634.7	50	17	3%
5	111.724	-4857.6	100	68	14%
6	322.742	-14032	150	279	57%
7	284.332	-12362	200	241	49%
8	253.480	-11020	300	210	43%
9	236.314	-10274	400	193	39%
10	230.641	-10027	500	187	38%
11	43.587	-1895.1	F_0		

Stabilization of DNA/PLL complexes (caged and complexed) with dextran sulfate



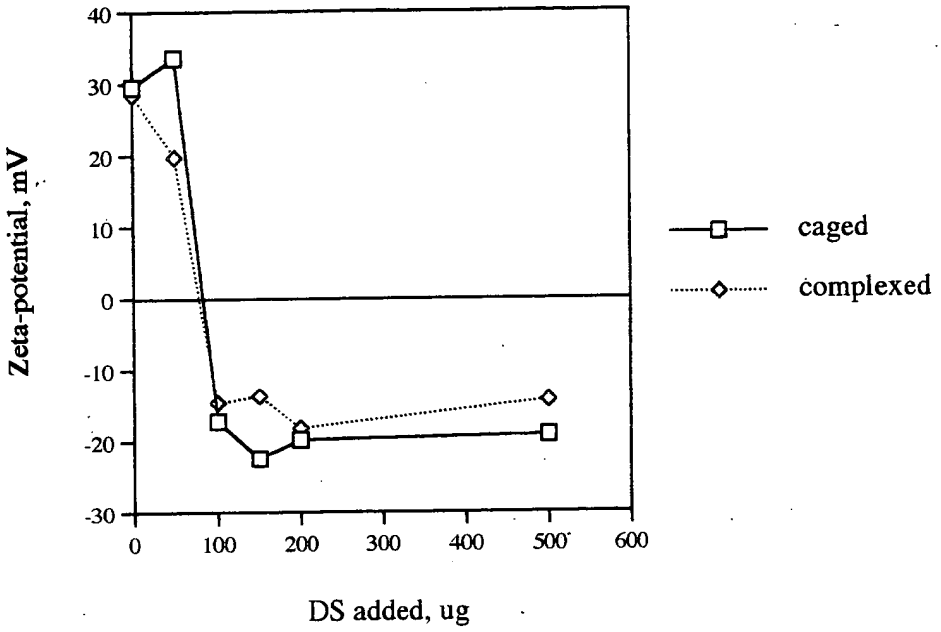
Condensation of DNA/PLL/DS complexes (caged and complexed)



Complexed DNA/PLL were prepared in the same conditions as for caged but w/o γ-Cinking with DTBP

ζ-potential is changed to opposite at 100 DS added.

Zeta-potential of DNA/PLL/DS complexes , no salt



PLL/DTBP(6,1.7) (Run 10)
VT
,DNA=20ug/ml, 25 mM HEPES, pH 8.0

Run	Zeta Potential (mV)	Half Width (mV)
1	31.34	3.67
2	33.02	2.11
3	26.96	3.57
4	39.37	1.96
5	30.17	2.31
6	24.25	2.10
7	26.53	1.95
8	22.45	2.10
9	29.20	1.85
10	29.55	2.96
Mean	29.28	2.46
Std. Error	1.51	0.22

PLL/DTBP(6,1.7)+50ugDS (Run 10)
VT
,DNA=20ug/ml, 25 mM HEPES, pH 8.0

Run	Zeta Potential (mV)	Half Width (mV)
1	49.36	3.06
2	44.33	1.83
3	37.00	1.80
4	33.83	3.36
5	39.11	2.34
6	27.81	1.81
7	28.67	4.53
8	11.79	1.82
9	36.92	1.84
10	28.00	3.19
Mean	33.68	2.56
Std. Error	3.30	0.30

PLL/DTBP(6,1.7)+100ugDS (Run 10)
VT
,DNA=20ug/ml, 25 mM HEPES, pH 8.0

Run	Zeta Potential (mV)	Half Width (mV)
1	-18.29	1.86
2	-8.36	1.87
3	-6.31	1.93
4	-14.52	1.93
5	-14.56	1.89
6	-21.63	1.83
7	-18.70	1.81
8	-25.67	2.50
9	-22.83	2.45
10	-21.59	2.07
Mean	-17.25	2.01
Std. Error	1.99	0.08

4	39.37	1.96
5	30.17	2.31
6	24.25	2.10
7	26.53	1.95
8	22.45	2.10
9	29.20	1.85
10	29.55	2.96
Mean	29.28	2.46
Std. Error	1.51	0.22

PLL/DTBP(6,1.7) (Run 10)
VT
,DNA=20ug/ml, 25 mM HEPES, pH 8.0

Run	Zeta Potential (mV)	Half Width (mV)
1	49.36	3.06
2	44.33	1.83
3	37.00	1.80
4	33.83	3.36
5	39.11	2.34
6	27.81	1.81
7	28.67	4.53
8	11.79	1.82
9	36.92	1.84
10	28.00	3.19
Mean	33.68	2.56
Std. Error	3.30	0.30

PLL/DTBP(6,1.7)+50ugDS (Run 10)
VT
,DNA=20ug/ml, 25 mM HEPES, pH 8.0

Run	Zeta Potential (mV)	Half Width (mV)
1	-18.29	1.86
2	-8.36	1.87
3	-6.31	1.93
4	-14.52	1.93
5	-14.56	1.89
6	-21.63	1.83
7	-18.70	1.81
8	-25.67	2.50
9	-22.83	2.45
10	-21.59	2.07
Mean	-17.25	2.01
Std. Error	1.99	0.08

PLL/DTBP(6,1.7)+100ugDS (Run 10)
VT
,DNA=20ug/ml, 25 mM HEPES, pH 8.0

Run	Zeta Potential (mV)	Half Width (mV)
1	-19.49	1.61
2	-30.43	3.32
3	-21.66	1.68
4	-20.73	1.63
5	-19.74	1.83
6	-21.84	3.94
7	-20.72	1.70
8	-30.38	2.06
9	-16.76	2.26
10	-22.71	1.92
Mean	-22.45	2.20
Std. Error	1.42	0.25

PLL/DTBP(6,1.7)+150ugDS (Run 10)
VT
,DNA=20ug/ml, 25 mM HEPES, pH 8.0

Run	Zeta Potential (mV)	Half Width (mV)
1	-19.39	3.39
2	-23.80	2.03
3	-15.61	1.90
4	-19.76	2.17
5	-17.92	2.75
6	-17.77	1.71
7	-22.13	4.28
8	-25.06	3.88
9	-18.99	1.92
10	-17.95	1.99
Mean	-19.84	2.60
Std. Error	0.93	0.29

PLL/DTBP(6,1.7)+200ugDS (Run 10)
VT
,DNA=20ug/ml, 25 mM HEPES, pH 8.0

Run	Zeta Potential (mV)	Half Width (mV)
1	-17.23	2.37
2	-8.34	1.96
3	-13.48	4.20
4	-23.75	1.84
5	-18.77	1.89
6	-15.59	4.34
7	-23.00	1.95
8	-23.10	2.04
9	-22.88	2.12
10	-25.96	1.84
Mean	-19.21	2.46
Std. Error	1.76	0.31

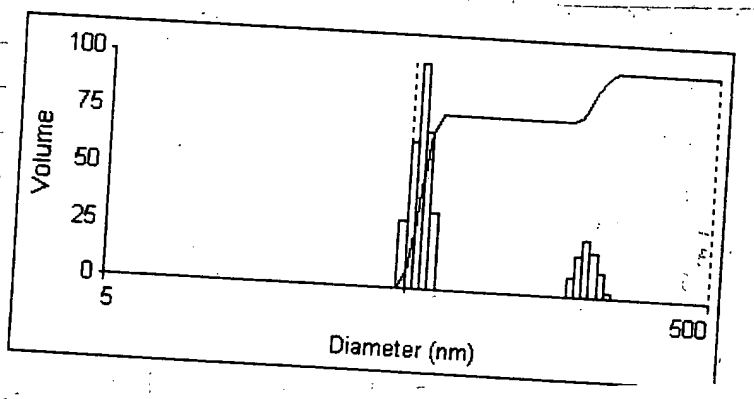
PLL/DTBP(6,1.7)+500ugDS (Run 10)
VT
,DNA=20ug/ml, 25 mM HEPES, pH 8.0

12/18/97 **Work on recharged ~~salt~~ DNA colloid (precipitation in salt)**

Samples prepared 12/17 (p77) were tested on precipitation upon addition of NaCl up to 150 mM
Was done with one sample 150 µg DS (close to neutrality point)

No.	I_{90} (700/700) FI	Conc.	
1	396.680	-17246	compl 150 µ DS
2	771.010	-33522	caged "
3	356.424	-15496	aft cent "
4	484.668	-21072	aft cent "
5	640.237	-27836	+ salt
6	667.412	-29017	+ salt
7	~~618.984~~	~~-26912~~	
8	~~681.295~~	~~-29621~~	
9	360.949	-15693	aft cent
10	400.766	-17424	aft cent.

There is some aggregates formed after each step but significant amounts of particles stays in solution after addition of salt and centrifugation



DNA/PLL (1:6) caged stabilized w 150 µ DS in 150 mM NaCl after centrifugation

Caged sample produced significant intens. (~1.2 M cps) after centrifugation

12/23/97 **Recharging the colloid with pMAA (not caged)**

In standard setups. Complexes were formed at DNA 50 µg/ml in 25 mM HEPES pH 8., PLL/DNA = 6:1, V = 0.5 ml (25 µg/50 µg)
→ then pMAA was add then each 0.5 ml was diluted to 1.5 ml with the same buffer
I_{90}, TRTD and ζ potential were measured

0 – 500γ pMAA was added to ~~each~~ each 2S DNA sample

No.	FI	Conc.
11	169.143	-7354.0

pMAA I_{50} (600/600)

No.	FI	Conc.
1	177.670	-7724.8
2	695.225	-30227
3	995.999	-43304
4	320.682	-13942
5	603.757	-26250
6	316.927	-13779
7	456.850	-19863
8	305.441	-13280

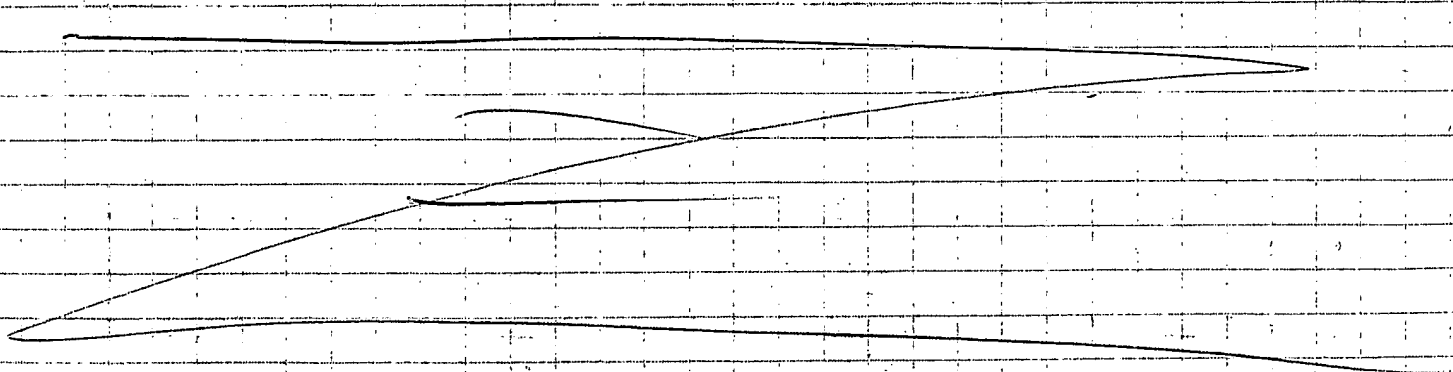
No.	FI	Conc.	pMAA	mt DNA
1	735.620	-31983	690	0
2	68.286	-2969.0	23	25
3	64.389	-2799.5	19	50
4	580.993	-25260	535	100
5	708.999	-30826	663	200
6	698.460	-30367	653	300
7	744.805	-32382	699	400
8	741.753	-32250	696	500
9	766.905	-33343	721	
10	45.911	-1996.1	F_0	

No.	FI	Conc.	2S
1	125.019	-5435.6	0
2	339.144	-14745	25γ
3	1001.452	-43541	50γ
4	964.944	-41954	100γ
5	644.407	-28017	200γ
6	634.971	-27607	300γ

I_{50}

No.	FI	Conc.	0 DNA	355
1	393.592	-17112		
2	54.019	-2348.7	25γ 0	16
3	45.936	-1997.2	50γ 25	7
4	47.624	-2070.6	100γ 50	9
5	359.647	-15636	100	321
6	225.945	-9823.7	200γ	187
7	206.946	-8997.7	300γ	168 47%

38 – F_0



12/30/97 Recharging the DNA/PLL colloid (uncaged)

repetition of experiments from previous page

TOTO

No.	FI	Conc.	pMAA		
1	13.291	-577.87	F_0		
2	894.401	-38886	F_∞	881	100
3	94.502	-4108.8	0	81	9.2
4	339.541	-14762	25	326	37.0
5	844.788	-36729	50	831	94.3
6	901.778	-39207	100	888	100.7
7	931.606	-40504	200	918	104.2
8	961.974	-41824	300	948	107.6
9	978.774	-42555	500	965	109.5

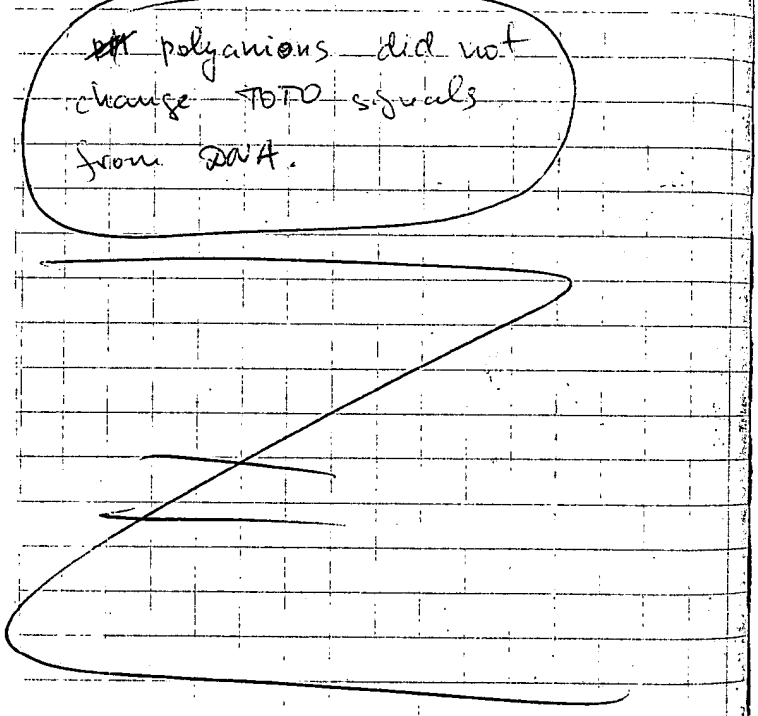
No.	FI	Conc.	pMAA -
1	14.718	-639.91	0
2	12.247	-532.48	25
3	11.329	-492.57	50
4	12.886	-560.26	100
5	12.353	-537.09	200
6	12.194	-530.17	300
7	12.591	-547.43	500

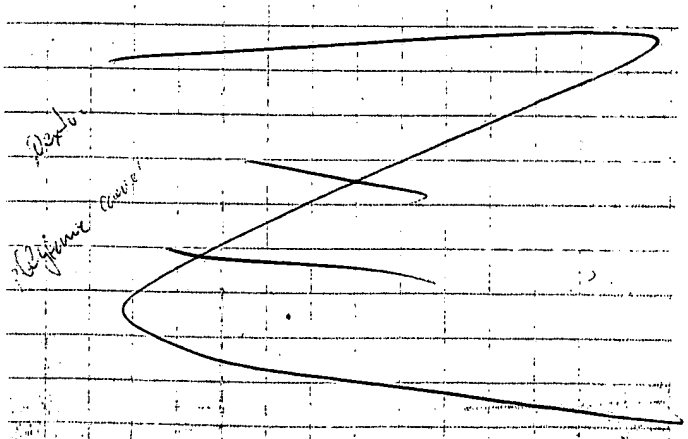
No.	FI	Conc.	DS		
1	29.793	-1295.3	F_0		
2	868.746	-37771	F_∞	856	100
3	86.448	-3758.6	0	74	8.6
4	62.691	-2725.7	25	50	5.8
5	158.887	-6908.1	50	146	17.0
6	854.383	-37147	100	842	98.4
7	433.794	-18860	200	421	49.2
8	371.326	-16144	300	359	41.9
9	345.736	-15032	500	333	38.9

No.	FI	Conc.	DS - DNA
1	15.943	-693.17	0
2	12.170	-529.13	25
3	11.950	-519.57	50
4	12.479	-542.57	100
5	12.135	-527.61	200
6	14.364	-624.52	300
7	12.913	-561.43	500

Conditions are the same as in p.80.

TOTO signals from pMAA alone and DS alone were measured. polyanions did not change TOTO signals from DNA.



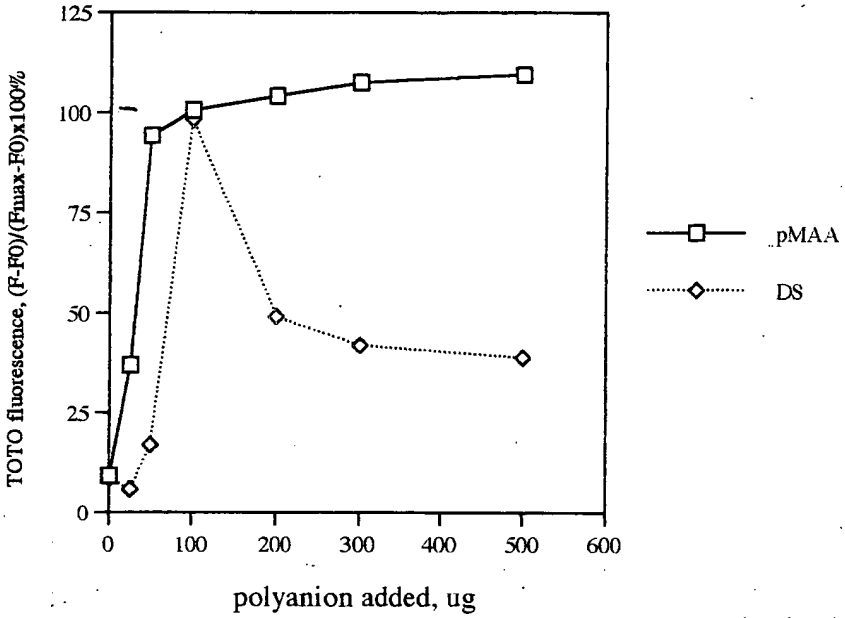


I 50 (100/600)

	FI	Conc.	ζ-potential	
	40.148	-6093.4	+ 0	DS
2	327.189	-14225	+ 25	
3	1008.335	-43840	+ 50	
4	753.784	-32773	— 100	
5	559.717	-24335	—	
6	408.500	-17760	—	
7	332.728	-14466	—	

No.	FI	Conc.		pMAA
1	337.505	-14674	+	
2	1008.335	-43840	+	
3	1008.335	-43840	—	
4	503.257	-21880	—	
5	203.894	-8865.0	— 100	
6	177.915	-7735.4	—	
7	135.729	-5901.3	—	

Condensation of DNA/PLL(1:6) upon addition of polyanions



1/6/98 Precipitation of DNA/PLL complex after recharging with polyanion.

The complex DNA/PLL (1:6) + 200 g DS was prepared as in p. 80 25 g / 95 g / 200 g in 0.5 ml 25mM HEPES, pH 8.0. Then it was diluted up to 1.5 ml.

0.5 ml of this solution (17 g/ml) was tested for I_{50}

No.		FI	Conc.	
1		173.291	-7534.4	DNA/PLL (1:6)
2	no salt	613.903	-26691	— " — + 200 g DS
3		219.280	-9533.9	DNA/PLL aff cent
4		541.387	-23538	— " + 200 g DS + aff. cent.
5		723.397	-31452	DNA/PLL (1:6) in salt.
6	150mM salt	984.784	-42816	— " + 200 g DS in salt
7		56.981	-2477.4	DNA/PLL in salt aff. cent.
8		588.275	-25577	— " + 200 g DS in salt aff cent.